Characterization of the interaction between the chaperone binding immunoglobulin protein (BiP) and the islet amyloid polypeptide allowing the inhibition of amyloid aggregation

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Introduction

Amyloid deposits in tissues are associated with various diseases, including light chain amyloidosis and transthyretin (TTR) amyloidoses, both being rare diseases leading to different forms of polyneuropathies. Before forming amyloid deposits, protein precursors undergo multitude conformations, some of which are cytotoxic, making the development of therapy against amyloidosis particularly difficult. The 70 kDa heat shock protein chaperones (HSP70) have been identified as potent inhibitors of amyloid formation[1].

The mechanism for facilitating proteins to fold HSP70 can go through the *foldase* mode, in the presence of ATP which causes a change in its conformation, or through the *holdase* mode, in the absence of ATP, which does not cause a change in its conformation[2].

Binding immunoglobulin Protein (BiP) is an HSP70 resident in the endoplasmic reticulum, the maturation site of islet amyloid polypeptide (IAPP), a peptide hormone whose aggregation is associated with type II diabetes.

Objective: Identify and characterize the domain of interaction between BiP, its subunits SBDα and SBDβ and IAPP in the absence of ATP to support the rational development of anti-amyloid therapeutics.

To follow the self-assembly of amyloid peptide, thioflavin T (ThT) is commonly used as it starts emitting fluorescence only when cross-β-sheet fibrils are present.

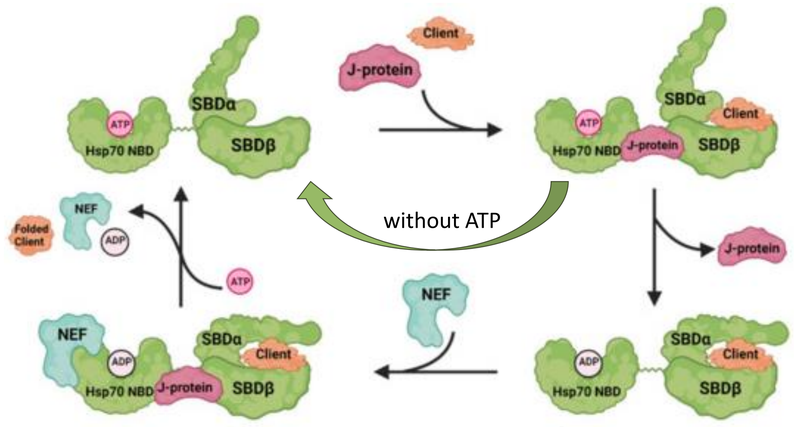
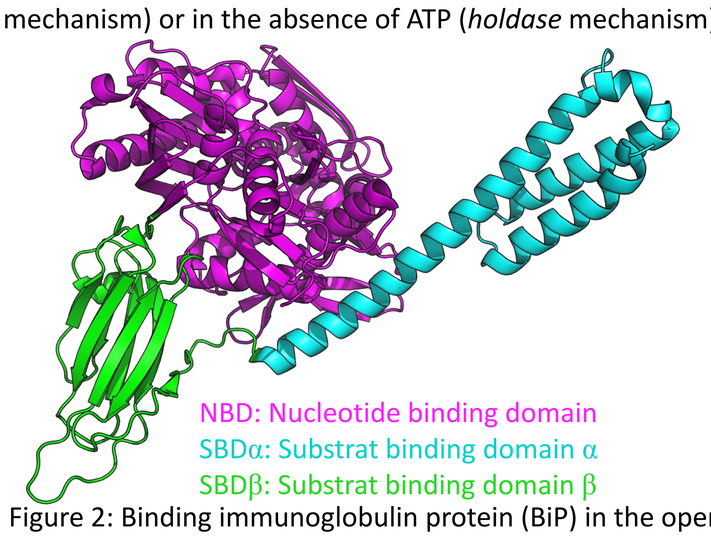


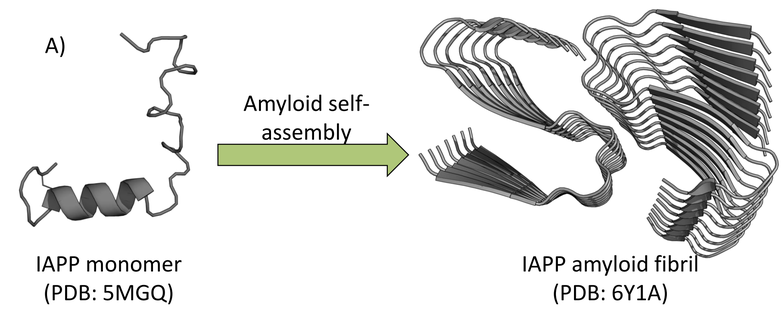
Figure 1: HSP70 mechanism[3]

HSP70s can help with folding in the presence of ATP (*foldase* mechanism) or in the absence of ATP (*holdase* mechanism)[2]



NBD: Nucleotide binding domain
SBDα: Substrat binding domain α
SBDβ: Substrat binding domain β

Figure 2: Binding immunoglobulin protein (BiP) in the open conformation (PDB: 6ASY)



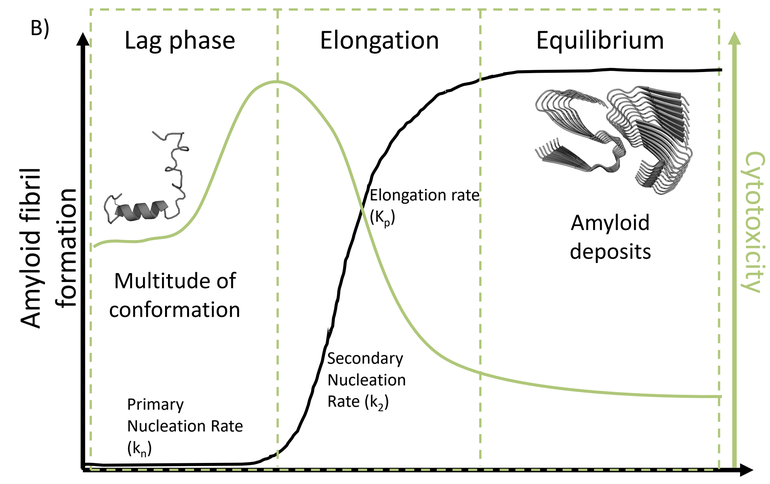


Figure 3: Amyloid self-assembly of IAPP A) IAPP switch from monomer to fibril, B) Process of amyloid formation and cytotoxicity generated by this self-assembly

Results

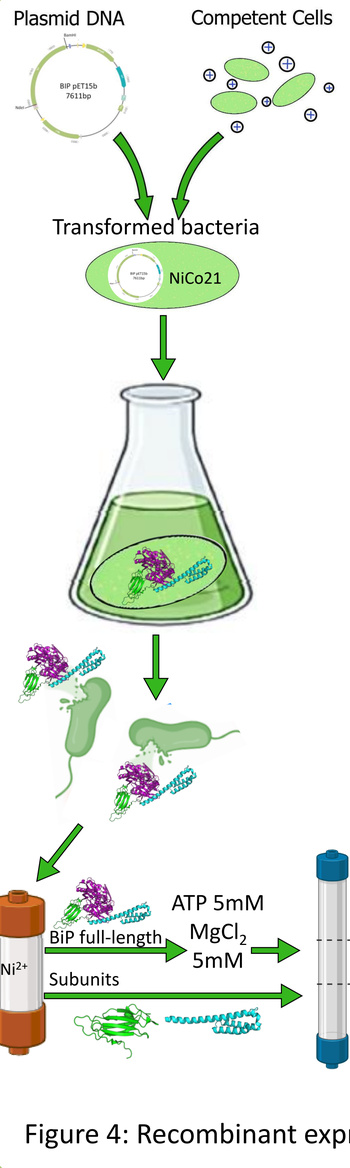


Figure 4: Recombinant expression and purification of BiP and its subunits (SBDα and SBDβ)

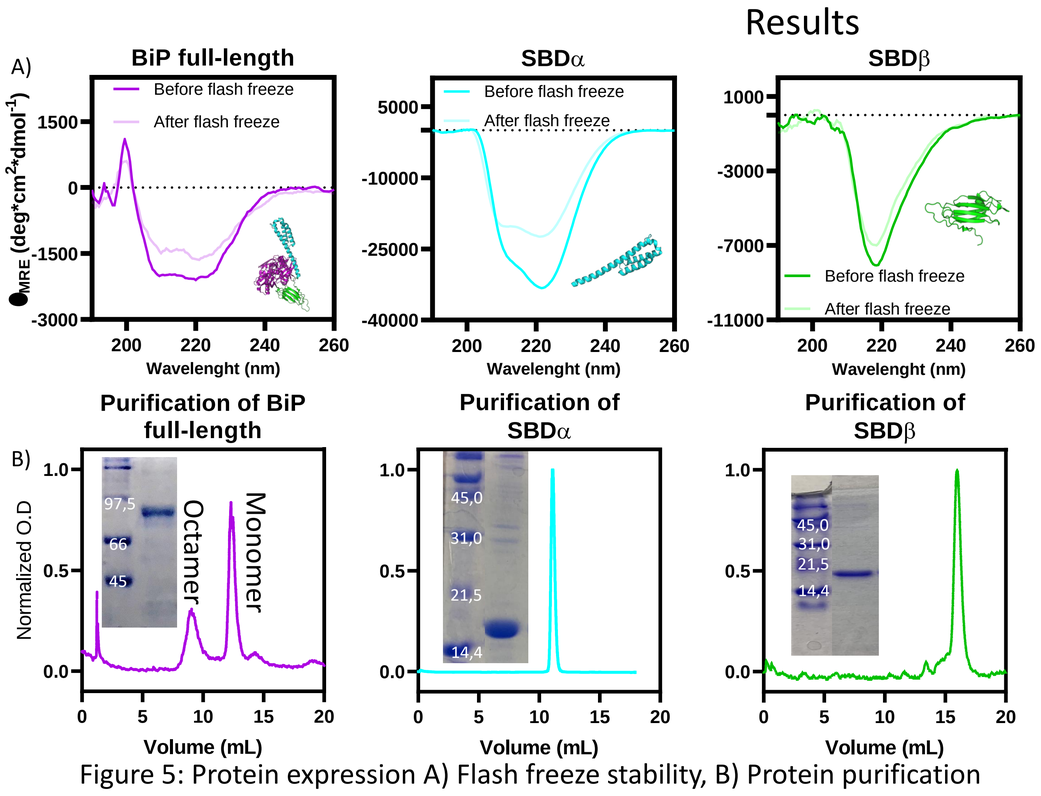


Figure 5: Protein expression A) Flash freeze stability, B) Protein purification

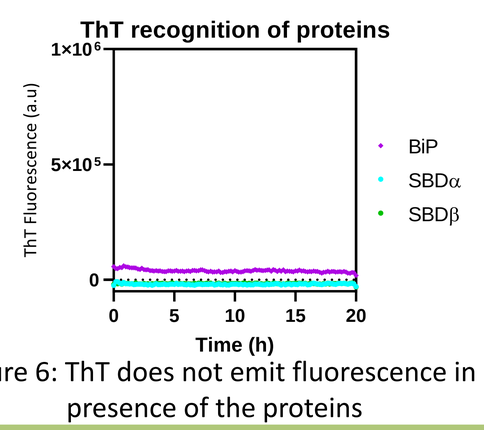
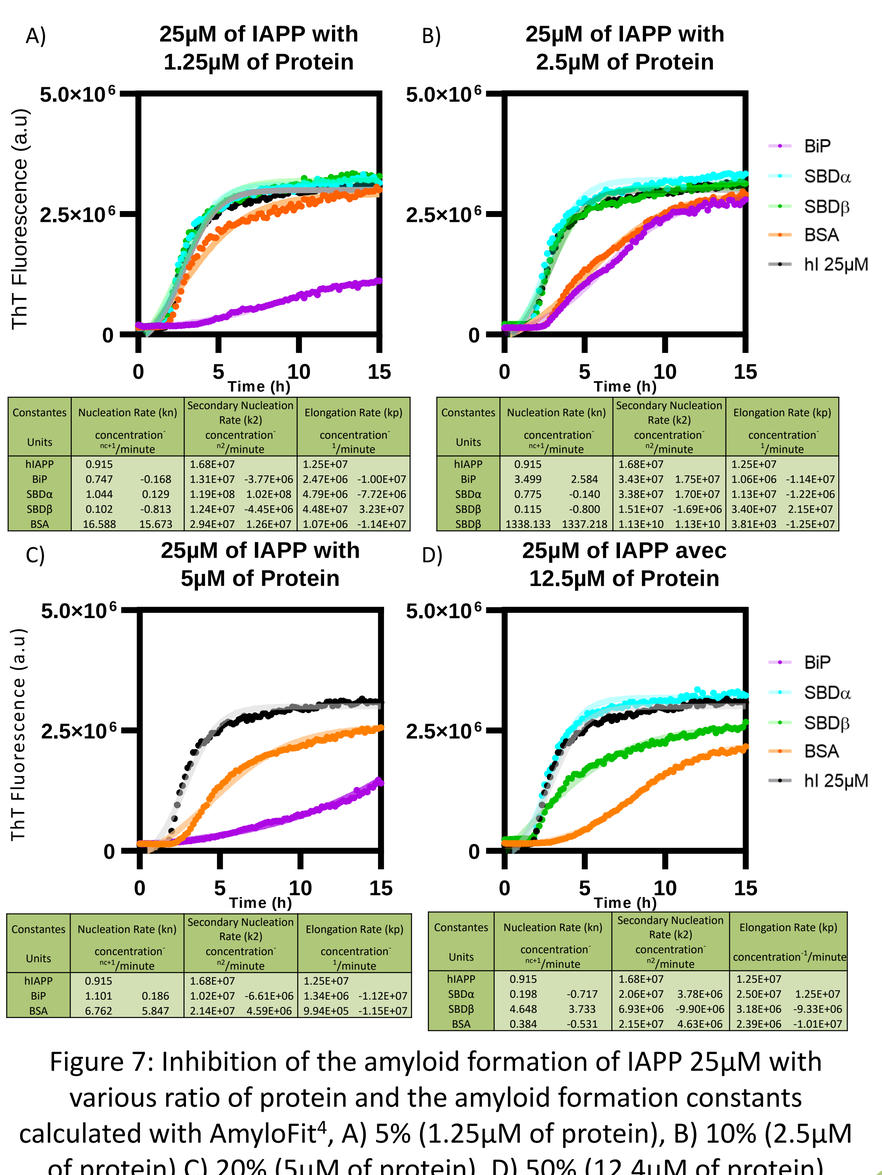


Figure 6: ThT does not emit fluorescence in presence of the proteins



Constantes / Units	Nucleation Rate (kn) concentration^{-nc+1}/minute		Secondary Nucleation Rate (k2) concentration^{-n2}/minute		Elongation Rate (kp) concentration^{-1}/minute	
hIAPP	0.915		1.68E+07		1.25E+07	
BiP	0.747	-0.168	1.31E+07	-3.77E+06	2.47E+06	-1.00E+07
SBDα	1.044	0.129	1.19E+08	1.02E+08	4.79E+06	-7.72E+06
SBDβ	0.102	-0.813	1.24E+07	-4.45E+06	4.48E+07	3.23E+07
BSA	16.588	15.673	2.94E+07	1.26E+07	1.07E+06	-1.14E+07

Constantes / Units	Nucleation Rate (kn) concentration^{-nc+1}/minute		Secondary Nucleation Rate (k2) concentration^{-n2}/minute		Elongation Rate (kp) concentration^{-1}/minute	
hIAPP	0.915		1.68E+07		1.25E+07	
BiP	3.499	2.584	3.43E+07	1.75E+07	1.06E+06	-1.14E+07
SBDα	0.775	-0.140	3.38E+07	1.70E+07	1.13E+07	-1.22E+06
SBDβ	0.115	-0.800	1.51E+07	-1.69E+06	3.40E+07	2.15E+07
BSA	1338.133	1337.218	1.13E+10	1.13E+10	3.81E+03	-1.25E+07

Constantes / Units	Nucleation Rate (kn) concentration^{-nc+1}/minute		Secondary Nucleation Rate (k2) concentration^{-n2}/minute		Elongation Rate (kp) concentration^{-1}/minute	
hIAPP	0.915		1.68E+07		1.25E+07	
BiP	1.101	0.186	1.02E+07	-6.61E+06	1.34E+06	-1.12E+07
BSA	6.762	5.847	2.14E+07	4.59E+06	9.94E+05	-1.15E+07

Constantes / Units	Nucleation Rate (kn) concentration^{-nc+1}/minute		Secondary Nucleation Rate (k2) concentration^{-n2}/minute		Elongation Rate (kp) concentration^{-1}/minute	
hIAPP	0.915		1.68E+07		1.25E+07	
SBDα	0.198	-0.717	2.06E+07	3.78E+06	2.50E+07	1.25E+07
SBDβ	4.648	3.733	6.93E+06	-9.90E+06	3.18E+06	-9.33E+06
BSA	0.384	-0.531	2.15E+07	4.63E+06	2.39E+06	-1.01E+07

Figure 7: Inhibition of the amyloid formation of IAPP 25µM with various ratio of protein and the amyloid formation constants calculated with AmyloFit[4], A) 5% (1.25µM of protein), B) 10% (2.5µM of protein) C) 20% (5µM of protein), D) 50% (12.4µM of protein)

Prediction of binding interactions

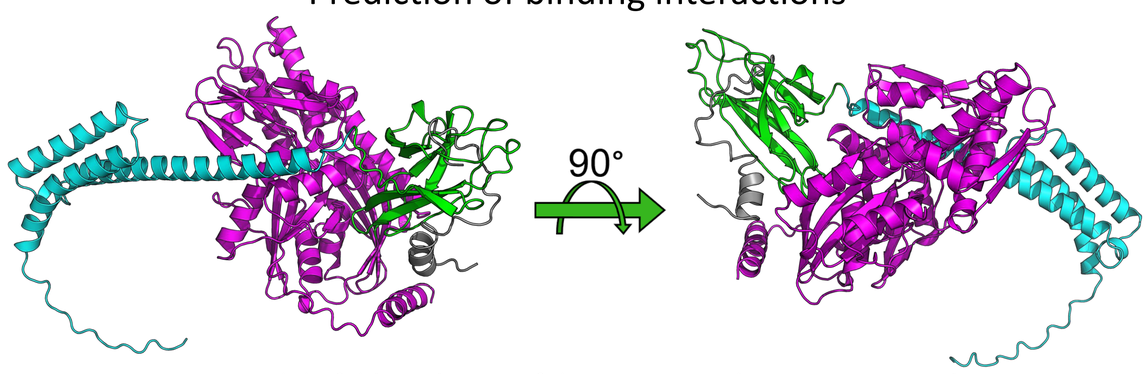


Figure 8: Alphafold prediction of the interaction between IAPP and BiP

Conclusions and perspectives

Figures 4, 5 and 6 show that the proteins are stable at -80°C, pure and do not react with ThT, allowing testing of their inhibiting impact on the self-assembly of IAPP.

As shown by the inhibition test (figure 7) and the predictive interaction generated by Alphafold (figure 8), the chaperone BiP needs all three domains to inhibit the self assembling process of IAPP. However, the ratio of BiP has an impact on the self-assembly of IAPP, as 5% and 20% (figure 7 A and C) inhibit the amyloid formation, but 10% (figure 7B) does not have the same impact.

More work is needed to understand how the ratio of BiP impact its inhibiting effect on the self-assembly of IAPP.

Further investigations on how this interaction occurs, such as nuclear magnetic resonance and mutation of BiP, will pave the way to the identification of novel therapeutics for amyloid-associated diseases.

References

[1]Collier, M. P., & Benesch, J. L. P., Cell Stress Chaperones (2020); [2]Chilukoti, N., et al., Biophys J, (2021); [3]Wang, J., et al., Gene, (2017); [4] G. Meisl et al., Nat. Protoc. (2016),

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